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# Methoxychlor Induces Estrogen-Like Alterations of Behavior and the Reproductive Tract in the Female Rat and Hamster: Effects on Sex Behavior, Running Wheel Activity, and Uterine Morphology

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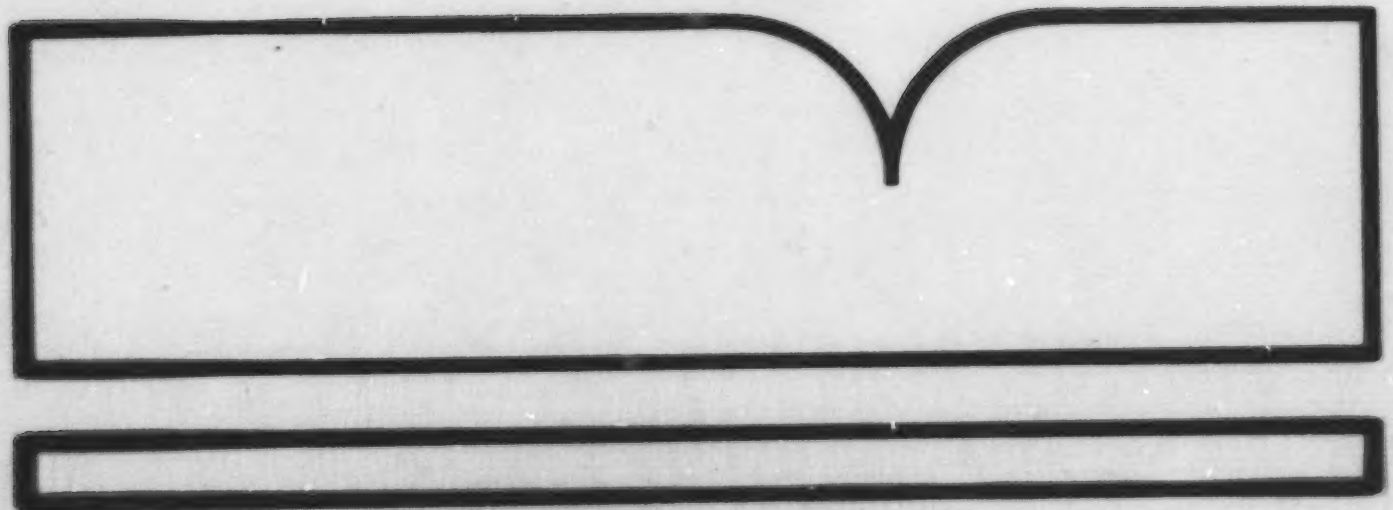

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In the first experiment, Fisher 344 female rats displayed much lower levels of estrogen-dependent running wheel activity (RWA) than did Long-Evans hooded or Sprague-Dawley female rats. However, RWA peaked after proestrus in all three stains and ovariectomy (OVX) reduced activity to equally low levels across the three strains. While Fisher 344 rats display statistically significant estrous cycle-dependent RWA, the cycles were of such low amplitude that it precluded using this strain in the assessment of toxicant-induced alterations of this behavior. In the second experiment, exposure to the weakly estrogenic pesticide methoxychlor induced estrogen-like alterations of RWA in intact and in ovx LE-hooded female rat. Methoxychlor induced high levels of acyclic RWA and persistent vaginal estrus in all intact treated females. Following ovariectomy, RWA declined precipitously in control but remained at high levels in methoxychlor-treated-ovx females. Additional estrogen-like effects noted in the treated females included alterations of reproductive tract morphology, pituitary prolactin content and attenuated body weight gain following ovariectomy. The observation of high levels of RWA in ovx-methoxychlor-treated female rats demonstrates that methoxychlor has estrogenic effects on the CNS in addition to its well documented effects on the reproductive tract.


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# Methoxychlor Induces Estrogen-like Alterations of Behavior and the Reproductive Tract in the Female Rat and Hamster: Effects on Sex Behavior, Running Wheel Activity, and Uterine Morphology

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Methoxychlor Induces Estrogen-like Alterations of Behavior and the Reproductive Tract in the Female Rat and Hamster: Effects on Sex Behavior, Running Wheel Activity, and Uterine Morphology. GRAY, L. E., JR., OSTBY, J. S., FERRELL, J. M., SIGMON, E. R., AND GOLDMAN, J. M. (1988). *Toxicol. Appl. Pharmacol.* **96,** 525–540. The current investigation was designed to determine if the pesticide methoxychlor (M) mimicked the effects of estrogen in the brain and on behavior. Running wheel activity (RWA) and sex behaviors were evaluated in this study because the role of estrogen in the regulation of these behaviors has been thoroughly established. M exposure at 400 mg/kg/day (90% pure) induced high levels of acyclic RWA and persistent vaginal estrus in the female rats. Following ovariectomy (ovx), RWA declined precipitously in controls but remained at high levels in M-treated-ovx females. M also produced estrogen-like alterations of the uterine endometrial epithelium, the ovary, and growth after ovx. In another study, ovx female rats were dosed with M at 200 mg/kg/day and then with progesterone (P). P acts as an antiestrogen and specifically suppresses estrogen-induced RWA. P blocks the synthesis of estrogen receptors in the CNS and reproductive tract but does not lower RWA induced by nonestrogenic mechanisms. After 14 days of M administration RWA was increased fourfold over the ovx-oil-treated females. Subsequently, P injections reduced RWA levels far below those seen when the ovx-M-treated rats were injected with oil. The P-induced decline represents a 95% inhibition of the M-induced increase in RWA. Subsequently, M-treated-ovx rats and hamsters were injected with P and tested for their ability to display reproductive behaviors when paired with a stud male. Female sexual behaviors are induced by the administration of estrogen followed by progesterone. In this study the M-treated females displayed reproductive behaviors, in contrast to the oil-treated rats and hamsters. The observation that the high levels of RWA induced by methoxychlor treatment in ovx rats can be suppressed by concurrent progesterone injections demonstrates that the increase in RWA is due to the estrogenic effects of methoxychlor on the CNS. The fact that methoxychlor, followed by P injections, induces behavioral estrus in the rat and hamster extends this estrogenicity to other areas in the CNS. © 1988 Academic Press, Inc.

Methoxychlor is a degradable organochlorine pesticide that possesses weakly estrogenic activity *in vivo* and *in vitro. In vitro,* purified methoxychlor is not active but must be di-demethylated by the liver to estrogenic metabolites; however, the technical grade material does possess some estrogenic activity (Bulger *et al.,* 1978). Both the technical grade methoxychlor and the di-demethylated metabolite markedly stimulate uterine ornithine decarboxylase activity and cause an increase in uterine weight *in vivo.* These effects are due to the fact that these compounds bind to the estrogen receptor and initiate the cascade of

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estrogenic effects on uterine physiology. As a consequence of this hormonal activity technical grade methoxychlor blocks fertility in the female rat. Receptivity is blocked in some females. Those that do mate become pseudopregnant because implantation is blocked (Bal and Mungkornkarn, 1978). The failure of implantation may be due, in part, to the fact that methoxychlor, like excessive levels of estradiol, inhibits the progesterone-induced decidualization of the uterus (Cummings and Gray, 1987).

While it has been clearly demonstrated *in vivo* and *in vitro* that technical grade methoxychlor mimics estrogen's effects on the female reproductive tract, it has not been shown that this pesticide can induce estrogenic effects on the CNS and behavior. While it would seem logical that methoxychlor would be estrogenic in the CNS as well, other reports suggest that this may not be the case. Two studies, one using the rat (Gellert and Wilson, 1979) and the other using the hamster (Gray *et al.*, 1985), reported that neonatal administration of this pesticide did not alter CNS sex differentiation as do other estrogenic chemicals. In addition, studies on the pesticide chlordecone, which has similar estrogenic activity on the reproductive tract, have failed to mimic the effects of estrogen on reproductive behavior (Uphouse *et al.*, 1986).

It has been known for over 60 years that running wheel activity (RWA) occurs at much higher levels in female rats than males and that females show a peak of activity every 4 or 5 days on the evening of behavioral estrus (lordosis behavior and mating occur only during estrus) (Wang, 1923; Slonaker, 1924; Young and Fish, 1945). RWA is markedly reduced when serum estradiol levels are lowered, due to either the physiological state (pregnancy, pseudopregnancy) of the female or following ovariectomy (ovx). A number of treatments that alter estrous cyclicity also reduce RWA. Some of these include thyroidectomy (Stern, 1970) and thyroid hormone administration (Stern and Murphy, 1972). The only chemical treatment that successfully restores RWA in ovx females to the high levels seen in intact females involves the subchronic administration of estrogenic substances. Daily injections of estradiol induce high levels of RWA but subcutaneous implantation of pellets is more effective (Young and Fish, 1945). Estradiol benzoate, implanted directly into the anterior hypothalamic–preoptic area, also greatly increases RWA while similar implants in the vicinity of the ventromedial hypothalamus depress food intake in spayed female rats (Wade, 1976). It is known that the hormone progesterone antagonizes the effects of estrogen on RWA, causing a decrease in RWA, by acting as an antiestrogen (Young and Fish, 1945; Rodier and Segal, 1977; Rodier, 1971; Gentry and Wade, 1976). Progesterone inhibits the synthesis of estradiol receptors in the CNS and reproductive tract thereby preventing the estradiol-receptor complex from reaching the nucleus of the target cell (Clark and Markaverich, 1988). Food deprivation is the only other treatment that stimulates RWA levels to those seen in estradiol-treated-ovx female rats. However, the development of this activity differs from that induced by estrogens in that it cannot be blocked by concurrent administration of progesterone (Rodier and Segal, 1977) or the synthetic antiestrogen MER-25 (Roy and Wade, 1975). We elected to use RWA as an index of the estrogenic effects of methoxychlor on the CNS in addition to the induction of behavioral estrus. The induction of estrus is more complex than RWA. Hormonal priming for behavioral receptivity requires the appropriate temporal administration of estradiol and progesterone (Morali and Beyer, 1979), while RWA is facilitated by estrogen alone (progesterone is inhibitory). For the observation of behavioral estrus we used both rats and hamsters because estrogen is essential in both species. Hamsters were not tested in the running wheels because this behavior is not regulated by ovarian secretions in this species as it is in the rat.

The first experiment was designed to describe the ontogeny of RWA in the Sprague–Dawley (SD), Long–Evans (LE)-hooded and Fischer-344 (F344) female rat. It has been reported that the F344 female does not display this estrogen-mediated behavior (Festing, 1979). A second experiment was designed to determine if methoxychlor altered RWA in the LE-hooded female rat at a dose that has previously been shown to cause infertility (Gray *et al.*, 1988). In the third experiment we replicated the observation that methoxychlor treatment caused an increase in RWA in ovx female rats and, in addition, we demonstrated that the methoxychlor-induced increase in RWA could be blocked by concurrent administration of progesterone. The results of the third and fourth experiments also demonstrate that methoxychlor mimics estrogen by inducing behavioral estrus in the ovx-progesterone-injected female rat and hamster.

## METHODS

### *The Development of Wheel Activity in Three Strains of Rats*

The first experiment was conducted in two blocks. In block one 10 females from each strain were placed in stainless-steel Wahman LC-34S running wheels (14 in. diameter × 4.25 in. wide) at weaning (Day 21 for SD and LE rats and Day 26 for F344). Five pregnant Fischer rats were received on Day 11 of gestation (Charles River, Kinston, NY) and female offspring from these litters were used in the current experiment. The LE-hooded (Charles River, Portage, MI) and SD (Charles River, Kinston) 21 day weanling females were placed in running wheels upon receipt from the supplier. Each running wheel cage had a home cage (25 × 15 × 12.5 cm) attached to the wheel and the door between them was left open so that females had free access to the running wheel. Purina rat chow and tap water were provided *ad libitum*. The room containing the wheels had a photoperiod of 12/12 with lights on at 6 AM EST and a temperature of 70°F. Females were checked daily for vaginal opening after which vaginal smears were taken until cyclicity was initiated. At Day 100, vaginal smears were recorded for a 12-day period to assure that all females were cycling and to relate this data to the concurrent RWA levels. At Day 127 females were ovariectomized under anesthesia (Nembutal ip at 40 mg/kg) and housed in the running wheels until Day 170. RWA data were collected continuously throughout the experiment. The running wheels were modified such that as the wheel rotates an armature interrupts a photocell beam with each rotation, the mechanical counter having been removed. Each photocell beam interruption was recorded by a microprocessor-based system (designed by George Ward, USEPA) and stored on a 24-hr basis. An average cycle for the activity data was calculated on a 4- or 5-day basis as appropriate such that each female had a single value for each phase of the cycle, including diestrus-one, diestrus-two, proestrus, and estrus. In addition, the post-ovx data from Day 148 to 167 were averaged from each female to determine if removal of the ovaries reduced RWA in all three strains. The log-transformed data were analyzed as a split plot design with one factor (strain) being between subjects and the other (cycle) being nested within subjects (Kirk, 1982). In the present study all *F*-values were calculated using PROC GLM in the SAS (SAS Users' Guide; version 5, 1985).

Due to the variable performance of the F344 females a replicate (block 2) was conducted in an attempt to more clearly delineate the RWA. At 29 days of age running data were collected until Day 131 on 12 F344 and 12 LE-hooded female rats. These females came from litters of pregnant rats (10 per strain). The F344 dams came from Charles River, Kinston, New York, and the LE-hooded dams were ordered from Charles River, Portage, Michigan. The data were inspected for 4- or 5-day cyclicity and an average cycle was calculated for each female, using data from Day 75 to 100, and analyzed as in block 1.

### *Determination of the Effects of Methoxychlor (400 mg/kg/day) on the Intact and ovx LE-Hooded rat*

In the second experiment 14 LE-hooded female rats (offspring of pregnant rats from Charles River, Portage, MI) were housed individually as in Experiment 1 and were dosed orally with corn oil (at 5 ml/kg) or methoxychlor (Sigma, technical grade II, 90%) in oil at 400 mg/kg/day starting at 22 days of age ($n = 7$ per group). Females were randomly assigned to treatment groups in a manner that provided equal means and variances in body weight on Day 22 (46.6 g). Starting at Day 26, animals were observed for onset of vaginal opening, and then vaginal smears were monitored throughout the study. RWA data were also collected daily throughout the experiment. At Day 64 four rats in each group were ovariectomized while the other three were sham operated. The ovaries taken from the ovx females were saved for histological examination. The females were returned to their wheels until 88 days of age. At this time the females were killed and the uterus, vagina, ovaries (shams),

kidney, liver, adrenals, pituitary, and Harderian glands were weighed. The uterus, vagina, and ovaries were fixed in Bouin's, embedded in paraffin, and stained with hematoxylin and eosin. Cross sections of both ovaries and uterine horns were examined by using light microscopy by Experimental Pathology Laboratories, Inc. (RTP, NC).

*Observation of RWA in ovx Rats Dosed with Methoxychlor (200 mg/kg/day)*

In the third experiment 20 60-day-old female LE-hooded rats (offspring from pregnant rats from Charles River, Raleigh, NC) were ovariectomized under anesthesia (Nembutal 40 mg/kg). At 80 days of age, 17 of these ovx females were housed continously in running wheels as in Experiments 1 and 2 with one exception; the lights went out at 1300 EDST. RWA, body weight, and vaginal smears were observed throughout the experiment. At 104 days of age, females were randomly assigned to one of two groups in a manner that provided each group with the same mean and variance in RWA during the pretreatment period. One group was dosed orally with corn oil ($n = 9$) at 5 ml/kg/day, while the other ($n = 8$) received methoxychlor at 200 mg/kg/day to determine if methoxychlor would increase RWA in the ovx female, as it did in Experiment 2. This treatment was administered for the rest of the study. The overall RWA and the increase in RWA over that seen prior to oral treatment (increase in RWA due to treatment = total RWA − RWA prior to oral treatment) were analyzed by a one-way ANOVA using PROC GLM on SAS.

*Observation of RWA in ovx Rats Dosed with Progesterone (SC) and Methoxychlor*

After 24 days of the oral treatment with methoxychlor (now 129 days of age) half the females ($n = 4$) were given daily sc injections of progesterone (P) in oil (4 mg/rat for 4 days, followed by 8 mg/rat for 10 days) while the others were injected with the vehicle (oil at 0.4 ml/day) ($n = 4$) (trial 1). After a recovery period of 11 days the injections were switched for trial 2, such that the P-injected females from trial 1 were administered oil in trial 2 and those given oil in trial 1 were injected with P in trial 2. If methoxychlor-induced RWA is due to its estrogen-like activity, then P should suppress the RWA to the pretreatment level seen after ovx. The absolute RWA levels, the decrease in activity, and the relative decrease in activity, expressed as a percentage of the methoxychlor-induced increase were analyzed by nested one-way ANOVAs on SAS (the effect of P on RWA versus oil is a within animal contrast with $n = 8$). (Percentage decrease = (RWA level during methoxychlor treatment − RWA during methoxychlor plus P or oil treatment) × 100/increase in RWA due to methoxychlor.)

*Observation of Sex Behavior in the Female Rat and Hamster*

*RATS.* Six days after the termination of the P and oil injections all rats from the fourth experiment received sc injections of 2 mg of P (0930, EDST) and were tested for sexual behaviors (between 1300 and 1500, EDST) with a stud male immediately after lights out (rats were observed under red light). The percentage of mounts accompanied by a lordosis (lordosis quotient) was calculated and the number of females displaying proceptive (darting) behavior was noted. The behavioral observation was terminated after five mounts had occurred or after 10 min had elapsed.

*Hamsters.* Pregnant hamsters were obtained from Engle Labs and their offspring were used in the present experiment. Females were housed in unisexual groups with littermates prior to this investigation. They were housed individually during the experiment. Hamsters were maintained on a 14/10 light/dark cycle (the long light phase of the photoperiod is essential for reproduction in this species) with the lights out at 1100 EST. Adult females (105 days old) were ovariectomized under Nembutal (100 mg/hamster or about 65 mg/kg). Six days later females were randomly assigned to treatments ($n = 15$/group) in a manner that provided each group with equal means and variances in body weight. The groups received oil (5 ml/kg), 200 mg/kg/day methoxychlor (technical grade) by gavage or estradiol benzoate (a positive control) at 0.1 mg in 0.1 ml of oil. The oil and methoxychlor groups were dosed daily for 2 weeks, while the positive controls were injected with estrogen only during the second and third weeks. On the fourth week only half of the positive controls were given estrogen. Estrogen injections were administered 2 days before testing. On the day of testing all hamsters received 0.5 mg of P (sc). Beginning at 1300 each treated female was paired with a male hamster for a period of 2 min. At this time the total number of seconds that the female maintained a lordosis (lordosis duration) posture was measured. Reproductive behavior tests were conducted on Days 6 (Week 1) and 13 of (Week 2) dosing and 6 and 13 days (Weeks 3 and 4) after dosing was terminated.

## RESULTS

### *Strain Differences in RWA in the Female Rat*

In the first experiment, RWA developed rapidly in some SD and LE females, with

TABLE 1

REPRODUCTIVE DEVELOPMENT AND RUNNING WHEEL ACTIVITY IN THE F344, LE-HOODED, AND SD FEMALE RAT

| | LE | SD | F344 |
|---|---|---|---|
| Block 1 | | | |
| No. rats | 10 | 8 | 10 |
| Age at vaginal opening (days) | 28.4 ± 0.2 | 31.7 ± 0.7 | 36.1 ± 0.5[a] |
| Age at first vaginal estrous cycle | 29.0 ± 0.5 | 31.8 ± 0.6 | 37.9 ± 1.0[a] |
| RWA levels (No. rev/day) | | | |
| Block 1 | | | |
| Diestrus 1 | 3,069 | 5,350 | 1,452 |
| Diestrus 2 | 4,349 | 6,849 | 1,745 |
| Diestrus 3 | — | — | 2,136 |
| Proestrus | 10,596 | 11,315 | 2,921[a] |
| Estrus | 3,260 | 5,487 | 1,531 |
| Ovariectomized | 926 | 1,045 | 769 |
| Block 2 | | | |
| No. rats | 11 | — | 12 |
| Diestrus 1 | 6,548 | — | 2,150[a] |
| Diestrus 2 | 6,611 | — | 2,564[a] |
| Diestrus 3 | 7,360 | — | 3,274[a] |
| Proestrus | 11,708 | — | 4,442[a] |
| Estrus | 6,141 | — | 2,240[a] |

*Note.* Values are means + SE. The data are from Experiment 1, blocks 1 and 2.

[a] $p < 0.01$ different from LE and SD.

peaks of activity coincident with vaginal opening and first estrus. Both of these pubertal indices were about 1 week later in the F344 rats than in the SD and LE strains ($p < 0.01$, Table 1). In general, RWA increased over the first 2 to 4 weeks, after which it remained fairly stable. LE and SD female rats had much higher levels of RWA than did the F344 rats ($p < 0.0001$). Although the cyclic changes in activity seen in the F344 females were relatively small, they did display significant increases in activity during proestrus. In each strain there were significant differences between females within that strain ($p < 0.01$) and statistically significant ($p < 0.0001$) cyclicity was present. In each strain the levels of activity during the phases of the cycle varied such that proestrus > estrus = diestrus > ovariectomy. Ovariectomy reduced RWA levels and abolished the cyclicity in each strain. The results of the second block confirmed those in the first; F344 female rats displayed much lower levels of RWA than did LE females, but the F344 did display 5-day activity cycles. While most of the SD (7/8, two died) and LE (16/21, one died) females had 4-day cycles (both blocks), almost all of the F344 rats (21/22) had 5-day cycles.

### *Behavioral, Morphological, and Endocrine Effects of Methoxychor (400 mg/kg/day) Administration in the Intact and ovx Female rat*

In the second experiment, methoxychlor treatment drastically altered behavior and morphology. On Day 26, vaginal opening had occurred in all the methoxychlor-treated females, while only one of the controls was open at this age ($p < 0.002$ by a Fisher's exact test). The control females displayed normal 4- or 5-day vaginal estrous cycles following vaginal opening but the vaginal smears of the methoxychlor-treated females were persistently cornified throughout the experiment.

RWA, pooled over 4-day intervals to eliminate the effect of the estrous cycle, increased linearly from Day 22 until Day 42 in a similar manner in the control and methoxychlor-treated females. During this time methoxychlor induced slightly higher RWA levels than that in control females (Fig. 1). Between 42 and 46 days the RWA of the methoxychlor-treated females increased more rapidly than that of controls and plateaued from Day 50 on at a higher level ($F(1,12) = 12.7$, $p < 0.01$, for overall main effect of treatment, and for the interaction, $F(9,108) = 2.35$, $p < 0.02$). This difference was due to the fact that the treated females displayed high levels of acyclic activity while the control females displayed cyclic RWA, with high levels of activity after proestrus and lower levels during estrus and diestrus (Table 2). Our attempts to identify cyclicity by pooling the data for each

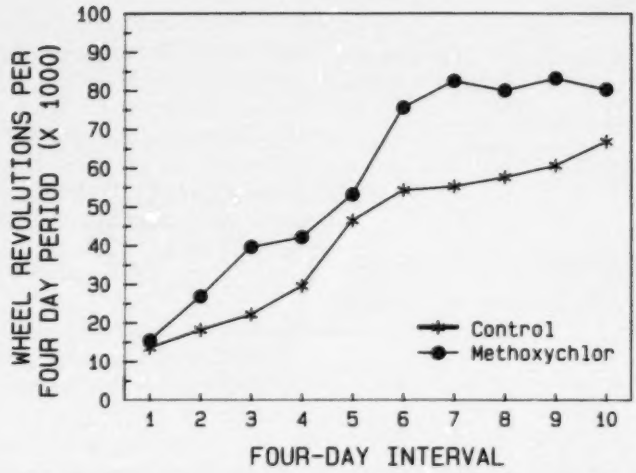



FIG. 1. In the second experiment methoxychlor treatment (400 mg/kg/day) increased the level of running wheel activity seen in the LE-hooded female rat. The overall treatment difference was significant ($F(1,12) = 12.7, p < 0.01$) and the treatment by time was also significant ($F(9,108) = 2.35, p < 0.02$). The data here have been pooled over 4-day intervals, starting at Day 22, to eliminate the effect of the estrous cycle on this behavior.

treated female on a 4- or 5-day basis were unsuccessful and for this reason, these data (Day 44 to 63) were further examined for cyclicity using spectral analysis techniques (spectra procedure available on the SAS, SAS User's Guide, 1979) as recommended by Gerall, 1967. This provides a more definitive estimate of the nature of cyclic changes present in sequential data such as the daily measurement of RWA. The periodograms, generated for each female by spectral analysis, represent the sum of squares in an analysis of variance sense for the decomposition of the process into two degree-of-freedom components. The $F$ statistic ($F(2,17)$), generated from the periodograms, indicated that the activity data of treated females were acyclic. The mean daily activity level, pooled over Days 44–65, of the treated females was significantly higher than that in the controls (Fig. 2). In addition, the "cycle amplitude" (calculated by subtracting the lowest score in each 5-day interval from the highest score over Days 44–65) was greater in the control females. In the acyclic females the cycle amplitude is actually a measure of the maximum day-to-day variation in RWA while in the cycling females this terminology is more accurate. The growth rate of methoxychlor-treated rats was significantly lower (8 to 9% less than that of controls) from Day 40 until ovariectomy on Day 65. At this time the controls weighted 193 g and the methoxychlor-treated females weighed 178 g ($p < 0.01$).

Following ovariectomy, activity levels of controls dropped dramatically to 4849 rev/day while activity in ovx-methoxychlor-treated females remained at a high level of 18,208 rev/day (Fig. 2). Following surgery, the ovx controls, now lacking estrogen, gained over 60 g (Fig. 3) while the growth of the methoxychlor-treated-ovx females resembled that of the intact (sham) females, gaining only about 20 g during the same time period.

As well as stimulating RWA, methoxychlor administration mimicked some of the effects of estrogen on the morphology of the

TABLE 2

THE EFFECTS OF DAILY TREATMENT WITH METHOXYCHLOR (400 mg/kg/day) ON THE REPRODUCTIVE PHYSIOLOGY AND BEHAVIOR OF THE FEMALE LE-HOODED RAT

| | Control (oil) | Methoxychlor |
|---|---|---|
| | (Running wheel activity: No. rev/day) | |
| Pre-ovx | | |
| No. rats | 7 | 7 |
| Proestrus | 20,666 | — |
| Estrus | 11,793 | — |
| Diestrus 1 | 12,713 | |
| Diestrus 2 | 13,835 | — |
| Pooled | 15,115 + 1,288 | 19,820 + 1,229[a] |
| Cycle amplitude | 10,884 + 1,327 | 6,948 + 532[c] |
| Post-ovx pooled over days 79–88 | | |
| Sham ($n$ = 3/group) | 14,744 + 1,619 | 17,172 + 740 |
| Ovx ($n$ = 4/group) | 4,849 + 671 | 18,208 + 1,664[c] |

*Note.* Values are means + SE. Rats were dosed from 22 to 88 days of age and ovariectomized or given sham operations on Day 65. All females were necropsied at 88 days of age. The date are from Experiment 2.

[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$.

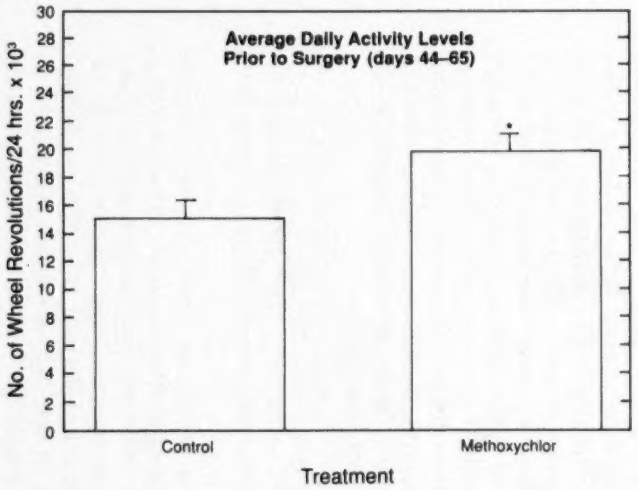
reproductive system (Table 3, Fig. 4). In the intact female, methoxychlor treatment caused atrophy of the ovary and uterus. Ovarian weight was reduced to 42% of control ($p < 0.0003$), and uterine ($p < 0.002$) weight was 72% of control. In the ovariectomized females methoxychlor treatment stimulated uterine ($p < 0.04$) weight, an estrogenic effect.

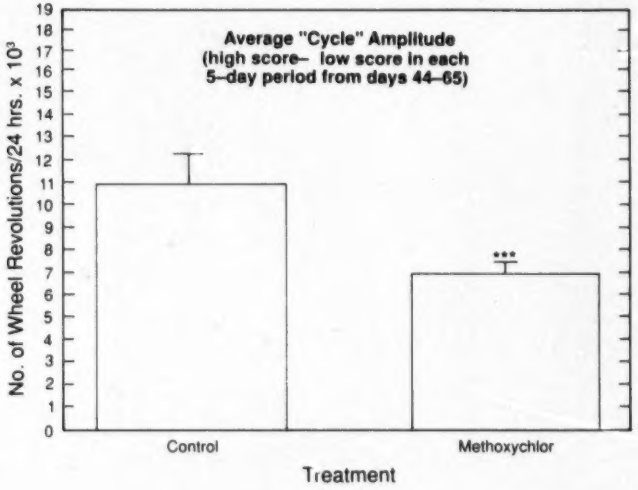
Histopathologic observations indicated treatment-related changes in the ovaries of both sham and ovariectomized rats given methoxychlor at 400 mg/kg/day. There was a decreased number of corpora lutea per cross section, an increase in atretic follicles and, an increase in cells in the interstitial tissue of the ovary. Treatment-related changes in the uterus consisted of slight to moderate hypertrophy of the endometrial epithelium. Instead of the low cuboidial epithelium present in the control rats, the uterus of methoxychlor-treated rats was lined by tall columnar epithelial cells.

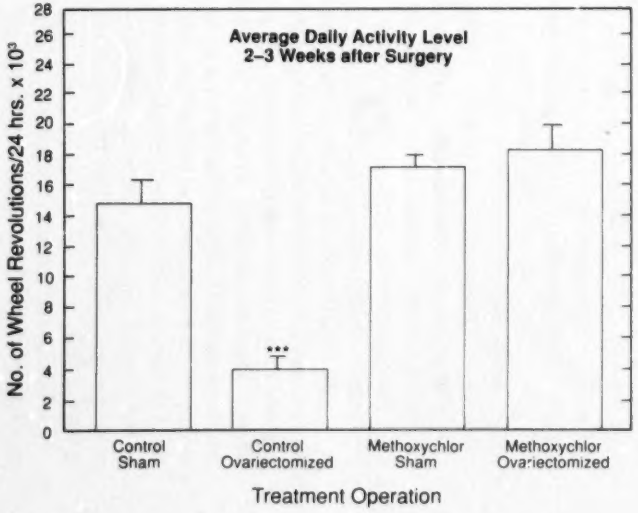
The serum and pituitaries were assayed for concentration of FSH, LH, and prolactin by RIA using the methods previously described by Goldman *et al.* (1986). Ovariectomy caused an increase in serum and pituitary concentration of LH and serum FSH, while serum prolactin levels were not affected (Table 4). Methoxychlor treatment did not reduce the ovx-induced increases in serum LH and FSH. However, methoxychlor treatment did reduce pituitary concentration of prolactin in the intact females, but increased it in the ovx females ($p < 0.001$, treatment by operation interaction).

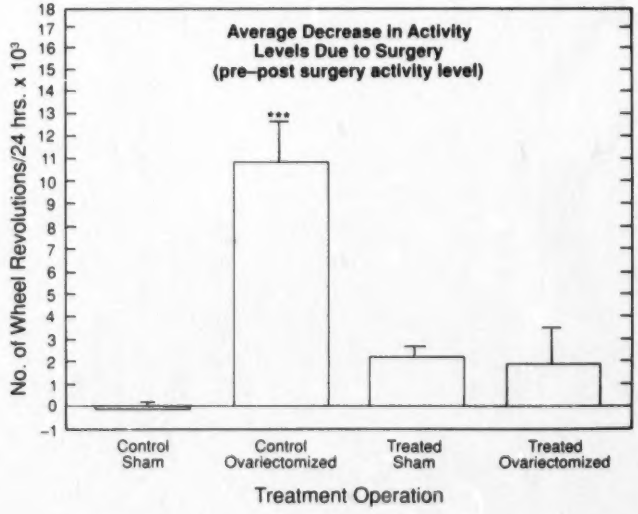
### *Effects of Methoxychlor (200 mg/kg/day) Administration on RWA in ovx Female Rats*

In the present experiment the 17 ovx females had RWA levels of 2883 rev/day (Fig. 5). Following oral dosing the RWA of the methoxychlor-treated females ($n$ = 8) increased significantly ($F = 69.5$, $p < 0.0001$) by 5830 to 8663 rev as compared to the females gavaged with oil ($n$ = 9) whose activity


Average Daily Activity Levels
Prior to Surgery (days 44–65)
No. of Wheel Revolutions/24 hrs. x 10³
Control
Methoxychlor
Treatment


Average "Cycle" Amplitude
(high score– low score in each
5–day period from days 44–65)
No. of Wheel Revolutions/24 hrs. x 10³
Control
Methoxychlor
Treatment


Average Daily Activity Level
2–3 Weeks after Surgery
No. of Wheel Revolutions/24 hrs. x 10³
Control Sham
Control Ovariectomized
Methoxychlor Sham
Methoxychlor Ovariectomized
Treatment Operation


Average Decrease in Activity
Levels Due to Surgery
(pre–post surgery activity level)
No. of Wheel Revolutions/24 hrs. x 10³
Control Sham
Control Ovariectomized
Treated Sham
Treated Ovariectomized
Treatment Operation

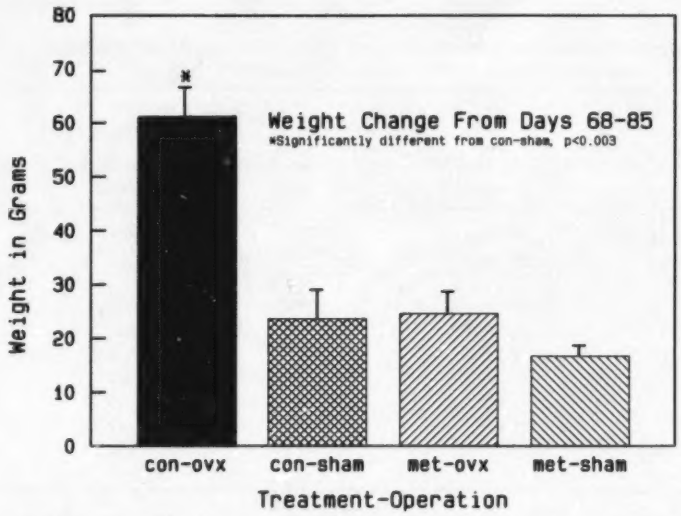



FIG. 3. Postsurgery growth. The effect of methoxychlor administration at 400 mg/kg/day on the growth of LE-hooded female rats between ovariectomy or sham operations at 65 days of age and necropsy at 88 days of age. Ovariectomized control females gained 40 g more than the sham-operated females, while methoxychlor treatment prevented this postovariectomy weight gain.

decreased by 780 to 1987 rev (not significant). The total RWA levels at this time also (8663 vs 1987) differed significantly between methoxychlor and oil treatments ($F = 59.9$, $p < 0.0001$, Fig. 5).

### *Inhibition of Methoxychlor-Induced RWA by Progesterone Injections*

Progesterone (4 mg) injections ovx-methoxychlor-treated rats displayed significantly lower RWA levels ($p < 0.01$) on injection Days 1–4 than did the oil-injected ovx-methoxychlor-treated rats (Fig. 5). Increasing the dose to 8 mg further depressed RWA on Days 5–7 ($p < 0.0001$) and Days 8–13 ($p < 0.0001$). When this decrease is expressed as a percentage of the methoxychlor-induced increase in RWA, P injection caused a 95% decrease while the oil-injected rats decreased by 24% (Fig. 6). Four days after P injections were terminated the RWA of these eight rats returned to their preinjection RWA levels.

FIG. 2. In the second experiment the activity levels of the rats had stabilized by 44 days of age after about 3 weeks in the running wheels. The average daily activity level prior to surgery (Days 44–65) (upper left) was significantly higher in the intact females dosed with methoxychlor at 400 mg/kg/day ($p < 0.05$). At this time the running activity of the methoxychlor rats fluctuated significantly less ($p < 0.001$) than that of the controls (upper right) because the controls behavior was cycling up and down regularly, while the methoxychlor rats, in contrast, maintained constant high levels of activity. The average daily activity level of the ovariectomized control females (2–3 weeks after surgery) was significantly lower ($p < 0.001$) than that of the other three groups (lower left). Methoxychlor administration prevented the decline in activity seen in the oil-treated control rats (lower right), ($p < 0.001$).

TABLE 3

THE EFFECTS OF OVARIECTOMY ON DAY 65 (OVX) AND CONTINUOUS METHOXYCHLOR TREATMENT (400 mg/kg/day; DAY 22–88) ON ORGAN WEIGHTS AT NECROPSY ON DAY 88

| | Treatment operation | | | |
|---|---|---|---|---|
| | Control sham | Methoxychlor sham | Control ovx | Methoxychlor ovx |
| Body at necropsy (g) | 219 (10.4)[a] | 196[b] | 251[c] | 199[b] |
| Growth after ovx (g) | 24 (8.4)[a] | 17[a] | 62[b] | 25[a] |
| Ovaries (mg) | 119 (7)[a] | 51[b] | — | — |
| Uterus (mg) | 265 (21)[a] | 191[b] | 95[c] | 130[d] |
| Vagina (mg) | 231 (35)[a] | 176[a,b] | 125[c] | 166[b,c] |
| Pituitary/ant (mg) | 7.2 (0.95)[a] | 5.2[b] | 9.8[c] | 6.5[a,b] |
| Kidney (mg) | 939 (47)[a] | 836[b] | 916[a] | 827[b] |
| Liver (g) | 11.4 (0.7)[a] | 11.2[a] | 11 9[a] | 10.8[a] |
| Adrenals (mg) | 67 (7)[a] | 65[a] | 59[a] | 66[a] |
| Harderian glands (mg) | 249 (18)[a] | 219[b] | 258[a] | 194[b] |
| No. rats | 3 | 3 | 4 | 4 |

*Note.* Values are means (pooled SD). Means with different letters differ significantly from one another. The standard errors can be calculated by dividing the SD by the square root of the sample size. These data were analyzed by two-way ANOVAs with main effects of oral treatment and the operation and an interaction.

### *Effect of Methoxychlor Administration on Sex Behavior in the Female Rat*

Methoxychlor-treated females ($n = 8$), injected with 2 mg of P, all displayed proceptive (darting) and receptive (lordosis) behaviors when paired with a stud male rat. These females had lordosis quotients (lordosis/mount ratio) of 1.0 while none of the oil-treated-ovx females displayed either behavior ($p = 0.000041$, by Fisher's exact test).

### *Effect of Methoxychlor Administration on Sex Behavior in the Female Hamster*

In the fourth experiment, using ovx hamsters, methoxychlor induced lordosis behavior in 2/14 (one was sick) after 6 days of dosing and 9/14 ($p < 0.001$ from oil 0/15, by Fishers exact test) after 13 days. One week after treatment was terminated 8/14 still displayed behavioral estrus, but by the second week of postdosing none of the females lordosed. At no time did any of the oil-treated females lordose. While methoxychlor did induce receptivity, it was less effective than was estradiol benzoate. During the tests (Weeks 2 and 3) when the positive control females had been pretreated with estrogen all 15/15 lordosed and they had lordosis durations of 104 sec out of the 2-min observation period. In contrast, the methoxychlor-treated females had lordosis durations of 38/120 sec on Week 1 (this was calculated using the values of only those females that lordosed), 71/120 sec during Week 2 of dosing, and 13/120 (Week 3) on recovery Week 1. Clearly the percentage of females lordosing and the duration of the behavior elicited by methoxychlor are much greater than zero, the effect seen in the oil-treated hamsters, but less robust than that seen in the positive controls (or in intact females). In the fourth week of testing the estrogen-treated females, dosed only on Weeks 2 and 3 but not 4, did not display receptivity as compared to those dosed on Weeks 2, 3, and 4, where 8/8 lordosed for 104/120 sec. Clearly, the effect of methoxychlor on sexual

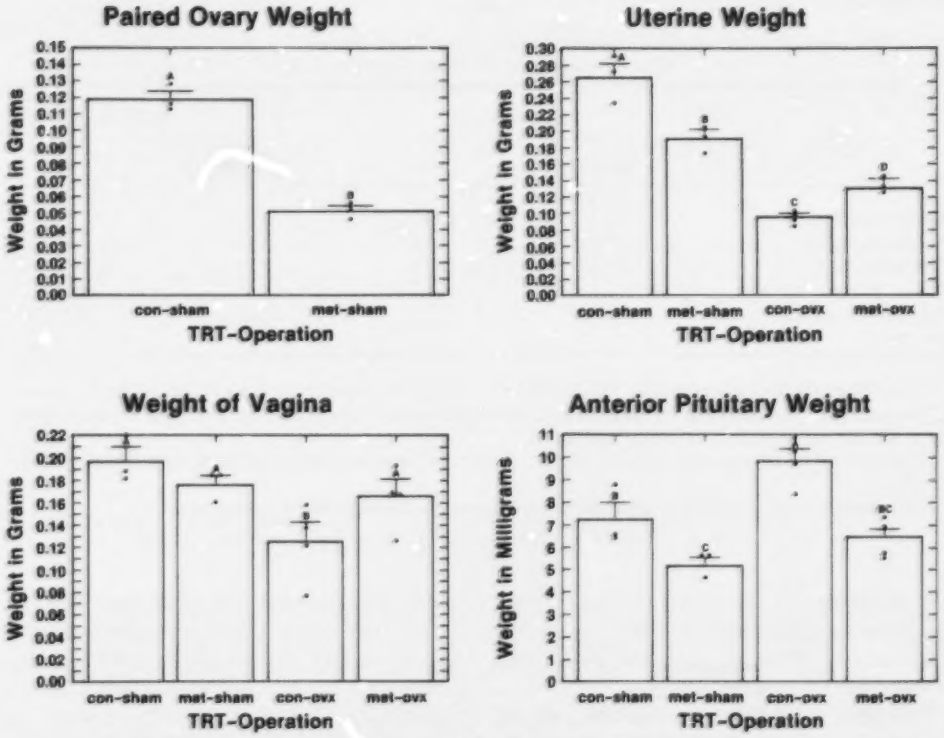



FIG. 4. The effects of ovariectomy and methoxychlor treatment on organ weights at necropsy. The reproductive organ weight data from the second experiment are presented here. These data were analyzed by two-way ANOVAs with an interaction. The effects on ovarian and uterine weight were highly significant, even though the sample sizes were small, due to the robust nature of the treatment effects. Methoxychlor administration at 400 mg/kg/day dramatically reduced ovarian weight in the sham-operated females, an effect that we have seen in other studies (Gray *et al.*, 1988). Uterine weight was similarly suppressed in the sham females by methoxychlor treatment. In the ovariectomized rats, methoxychlor stimulated uterine weight. Values are $\bar{X}$'s with +SE. Points represent individual values. Means with different letters are significantly different from one another.

receptivity persisted longer than did that of estradiol.

## DISCUSSION

In the present study methoxychlor treatment induced a large increase in estrogen-dependent running wheel activity in the ovariectomized female LE-hooded rat in both the second and the third experiments. Furthermore, this behavior was markedly inhibited by the concurrent administration of progesterone. The latter observation is a clear demonstration that the increase in RWA following methoxychlor treatment is a result of estrogenic activity in the CNS. Progesterone acts as an antiestrogen, blocking estrogen receptor synthesis, and P treatment has been shown to block only estrogen-induced RWA (as does the antiestrogen MER 25). P injection does not affect RWA induced by food deprivation. Methoxychlor treatment, followed

TABLE 4

THE EFFECTS OF OVARIECTOMY AND METHOXYCHLOR TREATMENT ON THE SERUM AND PITUITARY CONCENTRATION OF LH, FSH, AND PROLACTIN

| | Ovariectomy | | Sham | |
|---|---|---|---|---|
| | Control | Met | Control | Met |
| Serum FSH (ng/ml)[a] | 17.5 | 23.6 | 2.9 | 3.1 (6.8) |
| Serum LH (ng/ml)[a] | 2.78 | 3.45 | 0.34 | 0.21 (1.9) |
| Pit LH (μg/mg)[a] | 1.45 | 1.42 | 0.54 | 0.51 (0.12) |
| Serum prolactin (ng/ml) | 1.61 | 1.87 | 1.57 | 2.65 (0.55) |
| Pit prolactin (μg/mg) | 0.96 | 1.83[b] | 1.66 | 0.89[b] (0.30) |
| No. rats | 4 | 4 | 3 | 3 |

*Note.* Values are means (pooled SD). The standard error can be calculated by dividing the SD by the square root of the sample size. The data are from Experiment 2. These data were analyzed by two-way ANOVAs with main effects for oral treatment and operation and the interaction.

[a] The main effect of the operation was significant, and the ovx hormone level is significantly greater than sham ($p < 0.02$).

[b] The methoxychlor group differs significantly from control ($p < 0.01$) for the interaction term.

by progesterone injections, successfully induced behavioral estrus in both the rat and the hamster, a further demonstration of an estrogenic effect on the CNS.

In addition, it is likely that the growth rates of ovx-treated females resemble the intact females rather than the ovx controls due to an estrogen-like effect of methoxychlor on the CNS and food intake. It is well known that estrogenic compounds suppress growth through direct effects on regulatory behaviors, including reducing food intake and metabolic changes (Wade, 1976; Heywood and Wadsworth, 1980). In the second experiment the ovx-control females gained about 40 g more than the intact controls, while the ovx-methoxychlor and intact-methoxychlor females grew at the same rate.

Methoxychlor administration also produced estrogen-like effects on reproductive development, reporductive morphology, and histology. Methoxychlor accelerated vaginal opening, induced persistent vaginal cornification, and increased uterine weight in ovx females, effects which are well-known sensitive bioassays of estrogenicity (Jordan *et al.*, 1985). The increase in vaginal cornification reflects the estrogenic action of methoxychlor on the vaginal epithelium. In addition, the moderate hypertrophy of the endometrial epithelium and the decrease in the number of corpora lutea and increased number of atretic follicles of the ovary are also effects that can also be induced with long-term administration of estrogenic chemicals (Heywood and Wadsworth, 1980). For example, local application of estradiol to ovaries results in about a 50% reduction in the number of ovulations by inducing atresia of preovulatory follicles (Dierschke *et al.*, 1983).

While methoxychlor treatment induced estrogen-mediated RWA to high acyclic levels that equaled those seen on proestrus in control females, its effects on pituitary function were much less robust than those reported for long-term treatment with estradiol, DES, or chlordecone. After 16 weeks of estrogen administration Niwa *et al.* (1987) found that pituitaries were almost sixfold heavier and the mean serum prolactin levels of the estrogen-treated animals were 50 times higher than that of untreated-ovx females. DES produces

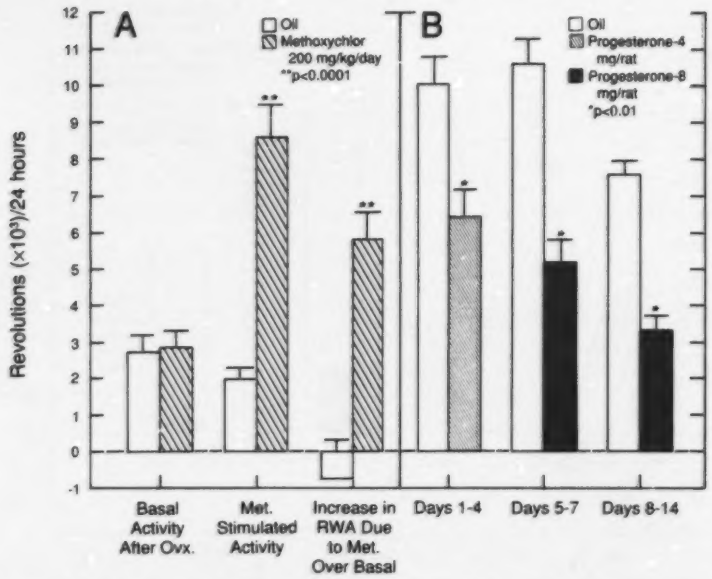



FIG. 5. These data are from the third experiment in which adult ovariectomized (ovx) females were dosed continously with methoxychlor at 200 mg/kg/day. Methoxychlor treatment significantly stimulated running wheel activity (A) over the pretreatment basal activity level ($p < 0.0001$). In the second phase of Experiment 3, starting after 24 days of oral dosing, the methoxychlor-treated-ovx rats were injected daily with progesterone (half in trial 1 and the other half in trial 2), an antiestrogen, and oil in the other trial. Progesterone was initially administered at 4 mg/rat on Days 1–4 of this phase of the experiment. The dose was then increased to 8 mg/rat for Days 5–14. The running wheel activity of the progesterone-injected rats was significantly lower than that of the oil-treated females (B).

similar effects in LE-hooded rats used in our lab (Cooper *et al.*, 1985). In addition, estradiol exerts a tonic inhibitory effect on serum LH during most of the estrous cycle and ovariectomy causes a pronounced increase in serum LH, which is readily suppressed by estradiol (Ramirez and McCann, 1963). In the present study pituitary weights were decreased rather than increased in the methoxychlor-treated females and, furthermore, methoxychlor treatment did not prevent the ovx-induced rise in serum FSH or LH, and had no effect on serum prolactin levels. The concentration of prolactin in the pituitary was the only measured endocrine parameter affected. Prolactin content in the pituitary was altered in a complex manner, being elevated by methoxychlor in ovx females but suppressed in methoxychlor-treated intact females. The effect of methoxychlor seen here on pituitary prolactin content in the female, and that reported by Goldman *et al.*, 1986, for the male rat, dosed with methoxychlor at 25 or 50 mg/kg/day for 10 weeks, represent relatively small changes (2-fold) in this parameter as compared to the 20-fold increase seen after 66 days of exposure to estradiol implants (Saade *et al.*, 1987). Goldman *et al.* (1986) postulated that this prolactinemic effect may represent an early component of the adverse action of methoxychlor on the reproductive system of the male rat.

The ability of methoxychlor to induce estrogen-mediated sexual behavior and RWA are in contrast to the behavioral effects reported for chlordecone, another estrogenic

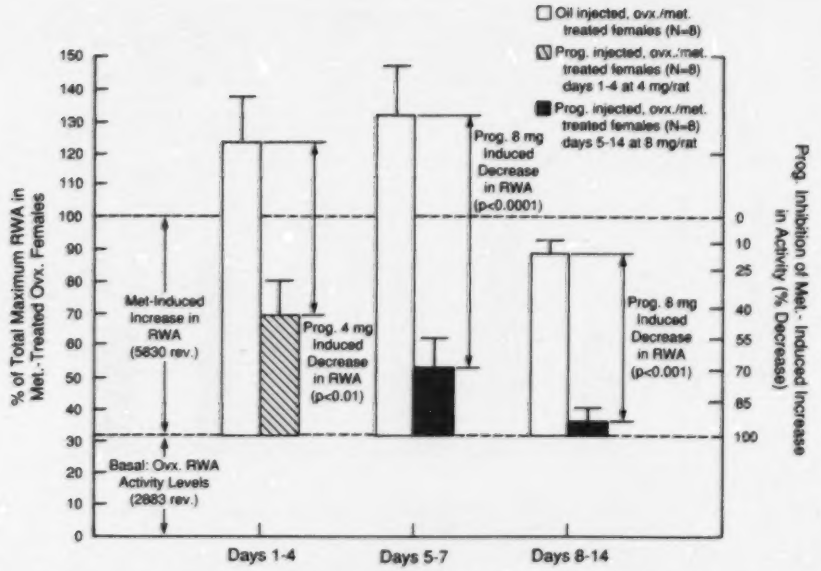

Oil injected, ovx./met. treated females (N=8)
Prog. injected, ovx./met. treated females (N=8) days 1-4 at 4 mg/rat
Prog. injected, ovx./met. treated females (N=8) days 5-14 at 8 mg/rat
% of Total Maximum RWA in Met.-Treated Ovx. Females
Prog. Inhibition of Met.-Induced Increase in Activity (% Decrease)
Met-Induced Increase in RWA (5830 rev.)
Prog. 4 mg Induced Decrease in RWA (p<0.01)
Prog. 8 mg Induced Decrease in RWA (p<0.0001)
Prog. 8 mg Induced Decrease in RWA (p<0.001)
Basal: Ovx. RWA Activity Levels (2883 rev.)
Days 1-4
Days 5-7
Days 8-14

FIG. 6. The effects of progesterone on methoxychlor-induced running wheel activity in ovariectomized female rats from the third study are examined in more detail. The methoxychlor-treated-ovx rats had total activity levels of about 8700 rev/24 hr (total activity = 100% of RWA). The basal activity levels of these rats prior to methoxychlor administration accounted for about 32% (2883 rev/24 hr) of the total activity. Methoxychlor treatment induced an increase of 5830 rev/24 hr, accounting for 68% of the total activity. If progesterone were 100% effective, then the administration of this antiestrogen would suppress activity by 5830 to the basal level of 2883 rev. The results indicate that progesterone injections in this study were 95% effective ($p < 0.001$ as compared to the activity level of the same methoxychlor-treated females during oil injections).

pesticide. Chlordecone resembles estrogen in altering pituitary function *in vivo* (Hudson *et al.*, 1984), producing vaginal cornification, increasing uterine weight, and competing for binding to the estrogen receptor, but fails to facilitate behavioral estrus in the female rat (Uphouse *et al.*, 1986). Furthermore, we have been unable to induce RWA in the ovariectomized female rat with chlordecone at doses (8 to 12 mg/kg/day for 7 days) that were sufficiently high to cause hyperreactivity to handling (Gray, unpublished). Uphouse *et al.* (1986) suggested that chlordecone acted as an antiestrogen, blocking lordosis, because it blocked estradiol-induced increases in progesterone receptors. In contrast to its lack of effect on estrogen-mediated behavior, chlordecone seems to mimic the effects of estradiol on the hypothalamic–pituitary axis to a greater degree than does methoxychlor. Hudson *et al.* (1984) found that implantation of DES or chlordecone for 4 weeks in ovariectomized females suppressed serum levels of LH and increased levels of prolactin as compared to ovx-controls. Similar effects were reported by Uphouse *et al.* (1984) and Reel and Lamb (1985). These investigators also reported that chlordecone, like methoxychlor, did not increase pituitary weights as do DES or estradiol.

In the first study, we found that the F344 female rat reaches puberty at about 1 week later than the Sprague–Dawley and LE-hooded strains and that most of the F344 fe-

males displayed lower levels of RWA than the females of the other two strains. However, the observation of peaks of running wheel activity after vaginal proestrus in the F344 strain, and the fact that RWA levels decline after ovariectomy, both indicate that RWA is influenced by ovarian hormones in the F344 in a manner that is at least qualitatively similar to the development of RWA in other strains of rats. The delay in puberty and lower RWA could indicate that either the F344 females have lower levels of circulating estradiol or they may have fewer steroid hormone receptors. Cooper (personal communication) has found that the F344 has low levels of serum estradiol on the morning of proestrus, providing support for the former hypothesis.

In conclusion, the present study clearly demonstrates that methoxychlor can promote estrogen-mediated behavior in the female rat and hamster. Similarly to estradiol, it also induces persistent vaginal cornification, uterine growth (in ovx females), and blocks ovarian function. Methoxychlor, however, reduces pituitary weight and does not affect LH secretion; thus, it does not precisely mimic the effects of estradiol or DES.

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## REFERENCES

BAL, H. S., AND MUNGKORNKARN, P. (1978). Chronic toxicity effects of methoxychlor on the reproductive system of the rat. *Proc. Int. Congr. Toxicol.* **1,** 446–447.

BULGER, W. H., MUCCITELLI, R. M., AND KUPFER, D. (1978). Studies on the in vivo and in vitro extrogenic activities of methoxychlor and its metabolities. Role of hepatic mono-oxygenase in methoxychlor activation. *Biochem. Pharmacol.* **27,** 2417–2423.

CLARK, J. H. AND MARKAVERICH, B. M. (1988). Actions of ovarian steroid hormones. In *The Physiology of Reproduction* (E. Knobil and J. Neill, *et al.*, Eds.), Vol. 1, Chap. 17, pp. 675–724. Raven Press, New York.

COOPER, R. L., GOLDMAN, J. M., AND GRAY, L. E. (1985). Assessment of pituitary function. *The Toxicologist* **5**(1), 182.

CUMMINGS, A. M., AND GRAY, L. E., JR. (1987). Methoxychlor affects the decidual cell reponse of the uterus but not other progestational parameters in female rats. *Toxicol. Appl. Pharmacol.* **90,** 330–336.

DIERSCHKE, D. J., BRAW, R. H., AND TSAFRIRI, A. (1983). Estradiol 17-b reduces number of ovulations in adult rats: Direct action on the ovary? *Biol. Reprod.* **29,** 1147–1154.

FESTING, F. W. (1979). Inbred strains. In *The Laboratory Rat* (H. J. Baker, J. R. Lindsey, and S. H. Weisbroth, Eds.), Vol. 1. Academic Press, New York.

GELLERT, R. J., AND WILSON, C. (1979). Reproductive function in rats exposed prenatally to pesticides and polychlorinated biphenyls. *Environ. Res.* **18,** 437–443.

GERALL, A. A. (1967). Effects of early postnatal androgen and estrogen injections on the estrous activity cycles and mating behavior of rats. *Anat. Rec.* **157,** 97–104.

GENTRY, R. T., AND WADE, G. N. (1976). Ses differences in sensitivity of food intake, body weight, and running-wheel activity to ovarian steriods in rats. *J. Comp. Physiol. Psychol.* **90**(8), 747–754.

GOLDMAN, J. M., COOPER, R. L., REHNBERG, G. L., HEIN, J. F., MCELROY, W. K., AND GRAY, L. E. JR. (1986). Effects of low subchronic doses of methoxychlor on the rat hypothalamic–pituitary reproductive axis. *Toxicol. Appl. Pharmacol.* **86,** 474–483.

GRAY, L. E., JR., FERRELL, J. M., AND OSTBY, J. (1985). Alteration of behavioral sex differentiation by exposure to estrogenic compounds during a critical neonatal period: Effects of zearalenone, methoxychlor, and estradiol in hamsters. *Toxicol. Appl. Pharmacol.* **80**(1), 127–136.

GRAY, L. E., JR., OSTBY, J., FERRELL, J., REHNBERG, G., LINDER, R., COOPER, R., GOLDMAN, J., SLOTT, V., AND LASKEY, J. (1988). A dose response analysis of methoxychlor-induced alterations of reproductive development and function in the rat. *Fundam. Appl. Toxicol.*, in press.

HEYWOOD, R., AND WADSWORTH, P. F. (1980). The experimental toxicology of estrogens. *Pharmacol. Ther.* **8,** 125–142.

HUDSON, P. M., YOSHIKAWA, K., ALI, S. F., LAMB, J. C., REEL, J. R., AND HONG, J. (1984). Estrogen-like activity of chlordecone (kepone) on the hypothalamo–pituitary axis: Effects on the pituitary enkephalin system. *Toxicol. Appl. Pharmacol.* **74,** 383–389.

JORDAN, V. C., MITTAL, S., GOSDEN, B., KOCH, R., AND

LIEBERMAN, M. E. (1985). Structure–activity relationships of estrogens. *Environ. Health Perspect.* **61,** 97–110.

KIRK, R. E. (1982). *Experimental Design: Procedures for the Behavioral Sciences,* 2nd ed. Brooks/Cole Publishing Co., Monterey, CA.

MORALI, G., AND BEYER, C. (1979). Neuroendocrine control of mammalian estrous behavior. In *Endocrine Control of Sexual Behavior* (C. Beyer, Ed.), pp. 33–75. Raven Press, New York.

NIWA, J., MINASE, T., HASHI, K., AND MORI, M. (1987). Immunohistochemical, electron microscopic, and morphometric studies of estrogen-induced rat prolactinomas after bromocriptine treatment. *Virchows Arch. B.* **53,** 89–96.

RAMIREZ, V. D., AND MCCANN, S. M. (1963). Comparison of the regulation luteinizing hormone (LH) secretion in immature and adult rats. *Endocrinology* **72,** 452–464.

REEL, JR., AND LAMB, J. C., IV. (1985). Reproductive toxicology of chlordecone (Kepone). In *Endocrine Toxicology* ed. (J. A. Thomas, K. S. Korach, and J. A. McLachlan, Eds.), pp357–392. Raven Press, New York.

RODIER, W. I., III (1971). Progestrone–estrogen interactions in the control of activity-wheel running in the female rat. *J. Comp Physiol. Psychol.* **74**(3), 365–373.

RODIER, W. I., III, AND SEGAL, S. (1977). The effect of progesterone on the activity-wheel running of ovariectomized rats. *Horm. and Behav.* **9,** 214–221.

ROY, E. J., AND WADE, G. N. (1975). Role of estrogens in androgen-induced spontaneous activity in male rats. *J. Comp. Physiol. Psychol.* **89**(6), 573–579.

SAADE, G., LONDON, D. R., AND CLAYTON, R. N. (1987). Effect of oestrogen treatment on gonadotroph function in adult male rats. *J. Endocrinol.* **114,** 95–101.

SAS Institute, Inc. (1979). *SAS User's Guide.* SAS Institute Inc., Cary, NC.

SAS Institute, Inc. (1985). *SAS User's Guide: Basics,* Version 5. SAS Institute Inc., Cary, NC.

SLONAKER, R. J. (1924). The effect of copulation, pregnancy, pseudopregnancy, and laction on the voluntary activity and food consumption of the albino rat. *Amer. J. Physiol.* **71,** 362–394.

STERN, J. J. (1970). The effects of thyroidectomy on the wheel running activity of female rats. *Physiol. Behav.* **5,** 1277–1279.

STERN, J. J., AND MURPHY, M. (1972). The effects of thyroxine and estradiol benzoate on wheel running activity in female rats. *Physiol. Behav.* **9,** 70–82.

UPHOUSE, L., MASON, G., AND HUNTER, V. (1984). Persistent vaginal estrus and serum hormones after chlordecone (kepone) treatment of adult female rats. *Toxicol. Appl. Pharmacol.* **72,** 177–186.

UPHOUSE, L., ECKOLS, K., SIERRA, V., KOLODZIEJ, M., AND BROWN, H. (1986). Failure of chlordecone (kepone) to induce behavioral estrus in adult ovariectomized rats. *Neurotoxicology* **7**(1), 127–142.

WADE, G. N. (1976). Sex hormones, regulatory behaviors, and body weight. In *Advances in the Study of Behavior* (J. S. Rosenblatt, R. A. Hinde, E. Shaw, and C. G. Beer, Eds.), Vol. 6. Academic Press, New York.

WANG, G. H. (1923). The relation between spontaneous activity and oestrus cycle in the white rat. *Comp. Psychol. Monogr.* **ii,** 1–27.

YOUNG, W. C., AND FISH, W. R. (1945). The ovarian hormones and spontaneous running activity in the female rat. *Endocrinology* **36,** 181–189.